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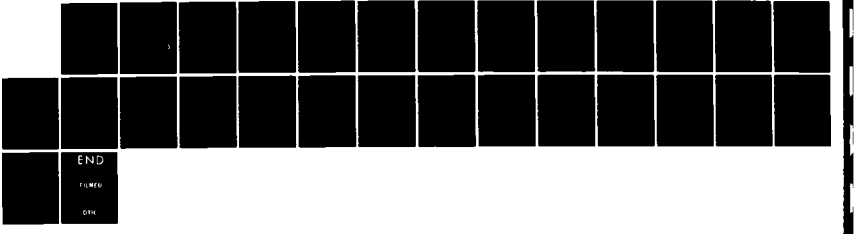

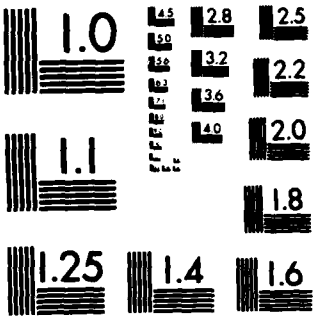

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Annual Report

IMMUNE DYSFUNCTIONS AND ABROGATION OF THE INFLAMMATORY RESPONSE BY ENVIRONMENTAL CHEMICALS

Richard G. Olsen
Department of Veterinary Pathobiology

For the Period
July 1, 1983 - June 30, 1984

AIR FORCE OFFICE OF SCIENTIFIC RESEARCH
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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Experiments done during FY 1983-1984 provide further evidence that UDMH enhances the immune response. The allogeneic mixed lymphocyte reaction (MLR) was enhanced by UDMH both when splenocytes in the reaction were incubated in the presence of UDMH, and when splenocytes from mice treated with UDMH were assayed in the MLR. The enhancement was most notable both in vivo and in vitro when the macrophage and B-cell enriched (stimulator) population was exposed to UDMH. This supports our hypothesis that macrophage suppressor activity is abrogated by UDMH, possibly by reduction of

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20. ABSTRACT (continued)

prostaglandin synthesis by the macrophage. Other experiments indicate that this effect is not due to alterations in intracellular cyclic nucleotide levels in splenocytes.

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Project Period:	July 1, 1983 - June 30, 1984
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Annual Scientific Report

I. Research Objectives

Fiscal year 1983-84 was quite productive for this project, as several areas of investigation into the mechanism of action of 1,1-dimethylhydrazine (UDMH) culminated into manuscript form (copy sent to Dr. Lind), another set of experiments confirmed a previously dubious observation (manuscript in preparation), and a third are of investigation was started which already shows promising results. Our specific aims as listed in the continuation proposal for FY 1983-84 were:

A. Determine in vitro effects of UDMH on distribution and numbers of immune cell subsets (Lyt 1 or helper T-cells, and Lyt 2, 3 or suppressor T-cells).

B. Determine the in vitro effects of UDMH on lymphocyte capping activity.

C. Determine the in vitro effects of UDMH on prostaglandin synthesis by peritoneal macrophages.

D. Determine the in vitro effects of UDMH on cyclic nucleotide levels (cAMP and cGMP) in peritoneal macrophages.

E. Determine the in vitro effects of UDMH on Ia antigen expression.

F. Determine the in vitro effects of UDMH on interleukin 1 (lymphocyte activating factor) synthesis by monocytes and interleukin 2 (T-cell growth factor) by lymphocytes.

Other aims which were conceived and completed or terminated during this time period include:

G. Determine the in vitro effects of UDMH on the syngeneic mixed lymphocyte reaction.

H. Determine the in vitro and in vivo effects of UDMH on the allogeneic mixed lymphocyte reaction, using enriched T-cell populations for responders and enriched B-cell populations for stimulators.

II. Status of Research

A. Determination of *in vitro* effects of UDMH on distribution and numbers of immune cell subsets (Lyt 1 or helper T-cells, and Lyt 2 or suppressor/cytotoxic T-cells.

These experiments were done to determine if UDMH alters the numbers of splenocytes expressing helper cell (Lyt 1.2) or suppressor/cytotoxic cell (Lyt 2.2) antigens. The experiments were initially done using fluorescein-conjugated reagents, and viewing by ultraviolet microscopy; the background, methodology, and results have been previously reported (see Final Report, July 1, 1982 - June 30, 1983). Because of the subjective nature of the methods and somewhat equivocal results, we proposed to repeat the experiments using a somewhat more quantitative method (enzyme-linked immunosorbent assay). However, we were recently awarded an instrumentation grant from the Department of Defense for a cytofluorograph. This instrument allows extremely accurate analysis and evaluation of any cell surface antigen, so all membrane marker experiments have been delayed until the cytofluorograph is installed (probably early 1985). The results of the initial experiments are reproduced in Table 1.

B. Determination of *in vitro* effects of UDMH on lymphocyte capping ability.

These experiments were also completed and reported in last year's final report. The following is excerpted from that report.

1. Background and methodology

"Capping" of mitogen or antigen by lymphocytes is a prerequisite to blast transformation and proliferation. The mitogen or antigen attaches to lymphocyte membrane receptors which then migrate to one location on the membrane before being internalized and processed. This can be visualized by using FITC-conjugated mitogen or antigen and visualizing the cells after a brief incubation period.

In these experiments, normal splenic lymphocytes were incubated with UDMH at 0 (control), 5, 10, 25, 50, 75, 100 or 200 µg/ml for 45 min., 2, 4, 24 or 48 hrs. FITC-conjugated concanavalin A was used for the last 45 minutes of the incubation period. They were then viewed through an ultraviolet microscope to determine the percentage of capped cells.

2. Results

UDMH did not affect the percent capping except after a 24hr incubation with 25-200 µg/ml, which resulted in decreased capping percentages (Table 2).

3. Discussion

The significance of the decreased capping percentage after 24 hr exposure to UDMH is questionable since a) both shorter and longer incubation periods did not affect capping; and b) alterations in the in vitro immune function assays noted previously occurred after only 2 hrs incubation with UDMH. In addition, one would expect, if anything, a suppressed immune response with decreased capping, not an enhanced response such as we observed, and the decrease would have to occur much earlier than 24 hrs since it is one of the initial events in the immune response. Thus, we concluded that UDMH does not exert its immunoenhancement effects by altering capping or receptor mobility.

C. Determination of the in vitro effects of UDMH on prostaglandin synthesis by peritoneal macrophages.

1. Background and methodology

One mechanism of regulation of immune responses has been shown to be by certain prostaglandins, particularly of the E series (PGE_1, PGE_2). PGE_2 alters the cyclic adenyl monophosphate:cyclic guanidyl monophosphate ratio in many cell types including lymphocytes which results in decreased lymphocyte activation and proliferation. PGI_2 (also called prostacyclin) is also

thought to inhibit the immune response. It has an extremely short half-life, so it is measured by its more stable but inactive metabolite, 6-keto-$PGF_{1\alpha}$. The source of PGs among immunocytes is probably exclusively newly activated macrophages. In our experiments murine resident peritoneal cells (RPC) were activated by 100 ng lipopolysaccharide and incubated in the presence of varying concentrations of UDMH for 24 or 48 hrs. The supernatant was then assayed for PGE_2 and 6-keto $PGF_{1\alpha}$ using a standard radioimmunoassay technique.

2. Results

There was a marked concentration related decrease in both PGE_2 and 6-keto $PGF_{1\alpha}$ levels after 24 hrs which was unrelated to decreased viability (Table 3). (Viability after 48 hrs, or above 100 ug/ml UDMH at 24 hrs, was significantly decreased.) Results are expressed as percent of control (untreated) cell PG production.

3. Discussion

The decrease in PGE_2 and PGI_2 production by peritoneal cells (mostly macrophages) could at least partly explain the immunoenhancement effects of UDMH. Since PGE_2 and PGI_2 suppress many immune functions, a decrease in their levels would allow for augmentation of the immune response. These results correlate well with our earlier reported findings that adherent splenocytes (composed largely of macrophages) also produce decreased levels of PGE_2 in the presence of UDMH.

D. Determination of the <u>in vitro</u> effects of UDMH on cyclic nucleotide levels in peritoneal macrophages and splenocytes.

1. Background and methodology

Many agents which influence immunocyte function do so by altering intracellular cyclic nucleotide levels (cyclid adenosine monophosphate or cAMP, and cyclic guanosine monophosphate, or cGMP). For instance, PGE_2 is thought

to inhibit mitogen-induced lymphocyte proliferation by inducing an increase in cellular cAMP. Conversely, mitogen-induced lymphocyte proliferation is associated with an increase in cellular cGMP.

Cyclic nucleotide levels were determined using a radioimmunoassay technique, identical in principle to the one used for PG determination. The sample is in the form of precipitated cellular protein. The initial series of experiments were done on splenocytes. The cells were incubated with 0, 5, 10, 25, 50, 75 or 100 µg/ml UDMH for 2 hrs, washed, then incubated with medium, lipopolysaccharide, or concanavalin A. After 10 minutes, 1 hr, 4 hrs or 24 hrs, the reaction was stopped by protein precipitation (using trichloracetic acid), and the sample was assayed for cAMP and cGMP levels. Since the results of these experiments were negative (see below), further experiments with peritoneal macrophages were not carried out.

2. Results

UDMH had no consistent or significant effect on either cAMP or cGMP levels after any of the time periods (Tables 4 and 5). Because of the expense and large numbers of cells needed to run these assays, and because no effects of UDMH on splenocytes were noted, further experiments with peritoneal macrophages were not carried out.

3. Discussion

These results would indicate that UMDH does not exert its immunoenhancement effect by altering cyclic nucleotide levels or cAMP:cGMP ratio in lymphocytes.

E. Determination of the <u>in vitro</u> effects on Ia antigen expression.

These experiments were first done using fluorescent reagents and ultraviolet microscopy, as already described for Lyt subset markers (IIA), and the results have already been reported.

Further experiments have been delayed until installation of the cytofluorograph, for the same reasons as stated in IIA. The results from the initial experiments are reproduced in Table 6.

F. Determination of the in vitro effects of UDMH on interleukin 1 and interleukin 2 synthesis and activity.

1. Background and methodology

Interleukin 1 (IL-1) (also called lymphocyte activating factor) is produced by activated macrophages (which occurs upon exposure to mitogens). IL-1 in turn induces interleukin 2 (IL-2) (also called T-cell growth factor) production by T-lymphocytes, which spurs blastogenesis by mitogen-primed lymphocytes. Interleukin 1 production and activity can be assayed by stimulating monocytes with lipopolysaccharide, then harvesting the supernatant (containing IL-1) and adding it to thymocyte cultures. Thymocyte proliferation is determined by measuring tritiated thymidine uptake in DNA. Interleukin 2 production and activity can be assayed by incubating purified T-cells with IL-1 (activated monocyte supernatant) and concanavalin A (con A), then harvesting this supernatant and adding it to cultures of con A-primed T-cells and measuring tritiated thymidine uptake.

It has been difficult to study the effects of compounds on interleukin production and activity, because it is not easy to isolate pure lymphocyte or pure macrophage populations from spleens or lymph nodes. IL-2 preparations are usually contaminated by IL-1, and vice-versa. The study of interleukins has been facilitated by the recent development of monoclonal cell lines which either produce pure IL-1 or IL-2 or are dependent on IL-1 or IL-2 for growth and survival. We have recently acquired some of these cell lines, and have just begun evaluating the effects of UDMH on their growth. The cell lines available are:

1) EL-4 thymoma cells, which produce high levels of IL-2 upon stimulation with phorbol-12-myristate-13-acetate (PMA).

2) CTLL-20, a lymphocyte cell line which is dependent upon IL-2 for proliferation. This cell line is used to monitor IL-2 production by other cell types (eg, EL-4 thymoma cells).

3) $P388D_1$, a macrophage cell line which secretes IL-1.

4) LBRM-33-1A5, a T-cell lymphoma cell line which secretes IL-2 only in the presence of IL-1 (used to monitor IL-1 production).

Our intention is to culture the IL-1 and IL-2 producer cell lines with various dilutions of UDMH, then wash them, culture them further and evaluate the supernatants for interleukin activity. Conversely, we also intend to evaluate the effects of UDMH on the growth of the interleukin-dependent cell lines in the presence of interleukins.

Preliminary experiments have been done to determine the effects of UDMH on EL-4 thymoma cell proliferation and viability. The growth rate and viability of the EL-4 thymoma cells when cultured with media containing 0 (control), 5, 10, 25, 50 and 75 µg/ml UDMH was monitored by removing 1 ml of cell suspension from each culture at 24 hr intervals for 96 hrs and determining the number of live cells present using the trypan blue dye exclusion test. UDMH caused a dose-related suppression of EL-4 thymoma cell proliferation when present in the cultures for 96 hrs. Significant suppression was observed at 48, 72 and 96 hrs when UDMH concentrations were 50 and 75 µg/ml. Concentrations of 25 and 10 µg/ml caused significant suppression at 48 and 72 hrs, respectively (Table 7).

Viability of the cells was not affected by the presence of the UDMH after 24 hrs of incubation. Concentrations of 50 and 75 µg UDMH/ml produced a significant decrease in viability beginning at 48 hrs and continuing for the duration of the incubation period (Table 8).

G. Determination of the *in vitro* effects of UDMH on the syngeneic mixed lymphocyte reaction (SMLR).

1. Background and methodology

The syngeneic mixed lymphocyte reaction (SMLR) is the proliferative response of T-cells to syngeneic (or autologous) non-T, Ia antigen-bearing cells. There is a great deal of controversy surrounding the immunologic significance of this reaction. Some evidence suggests the SMLR enhances the proliferation of suppressor and cytotoxic T-cells capable of regulating the immune response through a negative feedback mechanism. Other studies indicate that the T-cells activated in the SMLR are necessary for an immune response to conventional antigen. In mice exhibiting autoimmune diseases, the SMLR is absent or greatly decreased. Likewise, humans afflicted with Sjogren's Syndrome and systemic lupus erythmatosus (SLE) also have a depressed autologous mixed lymphocyte reaction. Since humans exposed to hydralazine and hydrazine sulfate develop a syndrome resembling SLE, it was decided to evaluate the effects of UDMH on the SMLR using murine splenocytes. Responder cells were enriched for T-lymphocytes by passage of splenocytes through a nylon wool column. Stimulator cells were enriched for B-cells by centrifugation through a discontinuous 35%-11% bovine serum albumin gradient; they were then inactivated by incubation with mitomycin C. The two cell populations were cultured together in microtiter plates for 5 days. Cell proliferation was evaluated by measurement of tritiated thymidine uptake into replicating DNA.

2. Results

Significant enhancement of the SMLR was observed at UDMH concentrations of 5, 10 and 25 µg/ml, and suppression was observed at 50 and 75 µg/ml (Table 9).

3. Discussion

The effects of UDMH on the syngeneic MLR have been studied. indicates that in vitro exposure of splenocytes to UDMH has a effect on this immune reaction, i.e., an enhancing effect at low trations, and a suppressive effect at higher concentrations. Althc study does not provide evidence for any mechanisms by which UDM these effects, it is possible the enhancement of the syngeneic MLR low UDMH concentrations could be attributed to decreased suppres activity. Direct cytotoxicity, metabolic interference, or possil deficiency might be factors involved in the suppression of the observed at higher concentrations of the chemical.

H. Determination of the in vitro and in vivo effects of UDMH on the al mixed lymphocyte reaction (MLR).

1. Background and methodology

The allogeneic MLR is similar in technique to the SMLR discusse except that a different strain of mouse is used for the responder ce these cells are reacting to histocompatibility antigens as wel antigens on the stimulator cells. The assay is customarily perfor unseparated spleen cell populations. We have looked at the effect on the MLR (both in vitro and in vivo) and have previously repc results. However, in reviewing that data we have noted that the re not correlate with our hypothesis based on other experiments t abrogates suppressor activity. We decided to repeat the MLR ex using a T-cell enriched responder population and a B-cell and m enriched stimulator cell population. Three different in vitro ex were done: 1) MLR with UDMH present throughout the duration of assay; 2) preincubation of the responder population with UDMH fc followed by washing and incubation with untreated stimulator cel

usual MLR assay; and 3) preincubation of the stimulator cell population with UDMH for 2 hrs, followed by washing and incubation with untreated responder cells in the usual MLR assay.

2. Results

When UDMH was present throughout the culture period, low concentrations of UDMH (5 and 10 μg/ml) resulted in an enhanced MLR, while higher concentrations (50 and 75 μg/ml) suppressed the response (Table 10). Pretreatment of the responder cell population with UDMH did not affect the MLR (Table 10), while pretreatment of the stimulator population resulted in an enhanced response at 25, 50, 75 and 100 μg/ml UDMH (Table 10).

3. Discussion

These results do support our hypothesis that UDMH enhances the immune response by abrogating suppressor activity of macrophages. UDMH-associated decrease in prostaglandin synthesis by macrophages (reported earlier) may be the mechanism by which this occurs.

4. In vivo effects of UDMH on the MLR.

Splenocytes from mice treated with UDMH were also tested in the MLR. The experiments have been completed and the results are now being analyzed statistically. The raw data indicate that UDMH treatment did result in enhanced MLR responses, as we predicted.

III. Written Publications (cumulative list)

A. Suppression of mitogen-induced blastogenesis of feline lymphocytes by in vitro incubation with carcinogenic nitrosamides. Tarr, M.J. and Olsen, R.G. Immunopharmacology 2:191-199, 1980.

B. Differential effects of hydrazine compounds on B- and T-cell immune function. Tarr, M.J. and Olsen, R.G. AGARD Conference Proceedings No. 309, Toxic Hazards in Aviation, B3-1-7, 1981.

C. In vivo and in vitro effects of 1,1-dimethylhydrazine on selected immune functions. Tarr, M.J., Olsen, R.G. and Jacobs, D.L. Immunopharmacology 4:139-147, 1982.

D. Species variation in susceptibility to methylnitrosourea-induced immunosuppression. Tarr, M.J. and Olsen, R.G. In press, J. Env. Path. and Toxicol. 1984.

E. Comparison of in vitro and in vivo immunotoxicology assays. Tarr, M.J., Olsen, R.G. and Jacobs, D.L. Annals N.Y. Acad. Sci. 407:469-471, 1983.

F. 1,1-Dimethylhydrazine Decrease Prostaglandin E_2 Production by Macrophages: A Possible Mechanism for Its Immunomodulatory Properties. Tarr, M.J., Olsen, R.G. and Fertel, R.H. Submitted to Intl. J. Immunopharm., 1984.

G. The Effects of 1,1-Dimethylhydrazine on the Murine Allogeneic Mixed Lymphocyte Response. McKown, B., Tarr, M.J. and Olsen, R.G. In preparation. To be submitted to Intl. J. Immunopharm.

H. Chemical Alteration of Host Susceptibility to Viral Infection. Tarr, M.J. In: Comparative Pathobiology of Viral Diseases, R.G. Olsen, ed. In press.

IV. Professional Personnel Associated with Research Effort

Richard G. Olsen, Ph.D., Principal Investigator

Departments of Veterinary Pathobiology, Microbiology (College of Biological Sciences), and Comprehensive Cancer Center, The Ohio State University, Columbus, Ohio 43210.

Melinda J. Tarr, D.V.M., Ph.D., Co-Investigator

Department of Veterinary Pathobiology, The Ohio State University, Columbus, Ohio 43210.

Grace Sutter, B.S., Research Assistant

Department of Veterinary Pathobiology, The Ohio State University, Columbus, Ohio 43210. Worked on this contract until August 12, 1983.

Brian Bowen, B.S., Graduate Research Associate

Mr. Bowen is working towards his Master of Science degree. He began working on this contract June 27, 1983.

Brenda McKown, B.S., M.S., Graduate Research Associate

Ms. McKown received her Master of Science degree in August, 1984, and was supported by this contract. Thesis title: The Effects of 1,1-Dimethylhydrazine on the Murine Allogeneic Mixed Lymphocyte Response. She is now employed by a pharmaceutical company.

V. Oral Presentations

A. Possible Explanations for the Immunoenhancement Effects of 1,1-Dimethylhydrazine. Tarr, M.J. and Olsen, R.G. Review of Air Force Sponsored Basic Research in Biomedical Sciences, University of California at Irvine, July 26-28, 1983.

Significance

The research performed during FY 1983-84 has fairly well confirmed our hypothesis that UDMH acts primarily on macrophages, and enhances the immune response by abrogating macrophage suppressor activity. One possible mechanism for this effect is decreased prostaglandin production by macrophages in the presence of UDMH, which we have reported earlier. Work during FY 84-85 will investigate other possible mechanisms as well as other effects of UDMH on macrophage function.

The work supported by this contract represents a good example of "applied basic immunology," in which new findings in the study of molecular immunology have been applied to evaluation of the immunotoxic effects of UDMH. This mechanistic approach to immunotoxicology is not evident in the current immuno-

toxicology literature, but many immunotoxicologists agree that this approach should be taken (Loose, Surv. Immunol. Res. 3:238-240, 1984), as it has many possible benefits, including augmentation of knowledge of basic immunology, development of possible prophylactic or therapeutic treatments for immunotoxicity, and development of simple screening assays for immunotoxic potential of drugs or environmental chemicals.

Table 1. Effects of UDMH on Lyt 1.2 and Lyt 2.2 expression on murine spleen cells

Concentration UDMH (µg/ml)	1 hr		2 hr		4 hr	
	Lyt 1.2	Lyt 2.2	Lyt 1.2	Lyt 2.2	Lyt 1.2	Lyt 2.2
0 (Control)	36.4±10.9	25.4±10.9	24.1±6.9	25.4±8.6	37.9±12.9	26.0±8.8
5	39.6±9.3	28.5±8.4	29.1±2.0[a]	28.8±7.1	36.9±14.2	25.3±5.6
10	31.1±13.1	27.9±8.2	37.6±9.3[b]	31.3±6.9	39.8±13.7	24.2±7.3
25	36.7±12.9	30.6±14.8	40.9±9.1[b]	24.1±7.0	34.5±15.1	27.5±9.3
50	33.4±12.0	30.0±8.7	35.5±7.5[b]	24.5±4.4	38.8±17.1	31.2±7.4
75	37.2±12.1	31.8±8.4	35.4±4.3[b]	23.1±8.0	42.3±13.7	26.5±6.7
100	43.5±13.0	33.6±4.7	35.8±4.9[b]	23.4±6.4	41.3±13.7	28.9±9.9
200	38.9±13.7	30.6±7.8	34.7±6.7[b]	25.1±10.5	42.0±14.7	30.1±7.2

[a] $p = 0.011$ (student "t" test).

[b] $p < .001$

Table 2. Effects of UDMH on capping of concanavalin A by murine splenocytes

Concentration UDMH (ug/ml)	Percent Capping				
	45 min	2 hr	4 hr	24 hr	48 hr
0 (control)	56.0±6.5	68.5±7.5	58.3±3.0	35.0±3.7	33.5±4.7
5	64.3±4.3	60.0±5.7	63.3±5.1	34.3±10.8	34.0±10.3
10	67.3±6.1	61.5±9.3	61.3±6.3	36.0±7.1	31.3±14.7
25	61.5±7.0	63.5±5.1	61.3±2.6	27.0±4.0[a]	30.5±10.4
50	56.0±4.8	61.3±3.7	57.3±4.8	27.0±6.1	36.0±12.2
75	59.5±8.5	61.0±5.0	61.5±7.9	23.8±5.0[a]	33.5±10.5
100	55.5±7.5	62.5±2.4	54.8±14.3	18.3±7.0[a]	39.3±13.8
200	56.0±2.9	56.0±8.3	48.0±9.9	16.7±10.0[a]	40.7±14.9

[a] $p < .025$ (student "t" test).

Table 3. Effects of UDMH on PGE_2 and 6-keto-$PGF_{1\alpha}$ (PGI_2) production by activated resident peritoneal macrophages

Concentration UDMH (µg/ml)	PGE_2 (% of control)	6-keto PGF_1 (% of control)
0 (control)	---	===
1	90.9%	ND
5	88.5%	ND
10	78.7%	82.8%
25	62.0%	ND
50	54.6%	55.8%
75	39.2%	ND
100	35.5%	42.8%

Table 4. Effects of UDMH on intracellular cyclic AMP levels in splenocytes[a]

Time after UDMH removal and medium or mitogen addition		Concentration UDMH (μg/ml)			
		0	10	50	100
	medium	3.88	3.69	4.31	3.61
10 min.	LPS	3.79	4.01	4.77	3.93
	con A	3.37	4.15	4.59	3.73
	medium	4.08	4.78	3.83	2.02
1 hr.	LPS	3.52	3.85	3.74	1.85
	con A	3.25	3.94	3.32	1.87
	medium	4.73	4.43	3.79	3.18
4 hr.	LPS	5.45	3.61	4.39	2.56
	con A	4.37	3.56	3.98	3.35
	medium	2.91	3.68	2.94	4.51
24 hr.	LPS	3.26	3.45	4.29	4.49
	con A	3.42	3.40	3.17	4.28

[a] Results expressed as fm/μg cellular protein.

Table 5. Effects of UDMH on intracellular cyclic GMP levels in splenocytes[a]

Time after UDMH removal and medium or mitogen addition		Concentration UDMH (µg/ml)			
		0	10	50	100
	medium	1.10	.99	1.18	1.31
10 min.	LPS	1.08	1.36	1.15	1.23
	con A	1.24	1.31	1.30	1.05
	medium	2.22	2.21	1.30	1.31
1 hr.	LPS	1.95	1.44	1.26	1.40
	con A	1.23	1.50	.94	1.61
	medium	1.64	1.90	2.99	3.07
4 hr.	LPS	1.79	2.09	3.10	3.41
	con A	2.11	2.47	2.84	3.17
	medium	2.36	3.97	4.26	2.57
24 hr.	LPS	2.39	3.10	3.38	2.10
	con A	3.31	3.27	2.94	2.12

[a] Results expressed as fm/µg cellular protein.

Table 6. Effects of 2 hr preincubation with UDMH on percent of adherent splenocytes expressing Ia antigen

Concentration UDMH (μg/ml)	Percent of cells expressing Ia antigen			
	24 hr. inc.	48 hr. inc.	72 hr. inc.	96 hr. inc.
0 (control)	71.9 ± 5.4	71.7 ± 4.4	69.4 ± 7.2	71.6 ± 5.3
5	77.8 ± 7.0[a]	76.2 ± 4.6	80.3 ± 4.8[b]	67.1 ± 6.0
10	69.7 ± 4.2	82.8 ± 2.5[c]	79.8 ± 7.4[d]	71.4 ± 8.6
50	71.2 ± 5.3	75.9 ± 4.6	74.2 ± 6.6	74.5 ± 8.9
100	80.0 ± 6.0[b]	79.5 ± 4.8[d]	77.0 ± 4.6	77.6 ± 7.0

[a] $p < .05$

[b] $p < .025$

[c] $p < .005$

[d] $p < .01$

Table 7. Effect of UDMH on EL-4 thymoma cell proliferation

Concentration UDMH (µg/ml)	Average number of viable cells per culture x 10^6				
	0	24	48	72	96
0 (control)	.1	.23 ± .10	.85 ± .08	1.42 ± .18	2.20 ± .38
5	.1	.18 ± .08	1.01 ± .25	1.31 ± .20	1.36 ± .32
10	.1	.19 ± .30	.66 ± .13	.79 ± .02*	1.10 ± .01
25	.1	.17 ± .08	.64 ± .04*	1.0 ± .12	1.16 ± .28
50	.1	.16 ± .06	.28 ± .08*	.42 ± .11*	.39 ± .11*
75	.1	.14 ± .03	.13 ± .01*	.26 ± .11*	.28 ± .11*

* P-value <.05 as determined by student "t" test; values compared to control values.

Table 8. Viability of EL-4 thymoma cells incubated with UDMH

Concentration UDMH (µg/ml)	Percent viability Hours in culture			
	24	48	72	96
0 (control)	63 ± 9	78 ± 1	81 ± 8	82 ± 11
5	53 ± 5	79 ± 3	82 ± 5	60 ± 8
10	60 ± 1	77 ± 3	70 ± 6	65 ± 11
25	63 ± 10	76 ± 4	71 ± 3	63 ± 1
50	60 ± 3	54 ± 3*	49 ± 11*	38 ± 0*
75	50 ± 14	48 ± 11*	42 ± 8*	44 ± 6*

* P-value $<.05$ as determined by student "t" test; values compared to control values obtained at same time.

Table 9. Effects of UDMH on the syngeneic mixed lymphocyte response[a]

UDMH Concentration µg/ml	% of Control Response (± S.D.)	P value[b]
0 (control)	-	
5	138 ± 48	$p < .001$
10	182 ± 50	$p < .001$
25	119 ± 21	$p < .01$
50	63 ± 66	$p < .005$
75	25 ± 21	$p < .001$

[a] UDMH was in the culture medium for the duration of the assay (5 days).

[b] P-value determined by paired "t" test.

Table 10. *In vitro* effects of UDMH on the allogeneic mixed l reaction.

Concentration UDMH (µg/ml)	UDMR present throughout MLR	UDMY pre-treatment of responders	UDMH treatme stimul
0 (control)	35,951 ± 14,725	34,805 ± 6,904	36,177 ±
5	43,400 ± 11,366[a]	34,671 ± 5,680	37,093 ±
10	40,636 ± 12,701[a]	36,485 ± 5,814	37,747 ±
25	33,923 ± 11,067	33,986 ± 6,663	39,669 ±
50	20,934 ± 7,476[b]	34,529 ± 6,858	43,012 ±
75	13,939 ± 6,492[b]	34,576 ± 4,072	43,529 ±
100	ND[c]	ND	41,152 ±

[a] Significantly greater than control according to Dunnett's Multiple Cc when $\alpha = .05$.

[b] Significantly less than control according to Dunnett's Multiple Compa when $\alpha = .05$.

[c] ND = not done.

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